



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2013

Cross-sectional study on fecal carriage of Enterobacteriaceae with resistance to extended-spectrum cephalosporins in primary care patients

Nüesch-Inderbinen, Magdalena ; Abgottspon, Helga ; Zurfluh, Katrin ; Nüesch, Hans-Jakob ; Stephan, Roger ; Hächler, Herbert

Abstract: The aim of this study was to gain knowledge of the local epidemiology of extended-spectrum cephalosporin-resistant bacteria in primary care patients in a Swiss community. Fecal swabs were obtained from 291 primary care patients. Phenotyping and genotyping methods were used for further characterization of the isolates. Risk factors associated with carriage of β -lactam-resistant strains were determined. Extended-spectrum cephalosporin-resistant Enterobacteriaceae were detected in 15 (5.2%) of the primary care patients. Thirteen isolates were CTX-M producers, one produced SHV-12, and three carried CMY-2. The pathogenic pandemic clone *Escherichia coli* ST131 was detected in 26.6% of the patients. Two patients (13.3%) carried two distinct strains simultaneously. There was a statistically significant risk of carriage of resistant strains for persons with a history of antibiotic therapy 4 months before sampling ($p=0.05$), markedly for therapy with β -lactam ($p=0.01$). Age, gender, or history of hospitalization 4 months before sampling was not a risk factor for the acquisition of resistant bacteria in the analyzed patients. The relatively low prevalence of extended-spectrum cephalosporin-resistant strains in the community reflects the nationwide restrictive policy of antibiotic prescription as well as local implementation thereof. Nevertheless, our study shows that a potent antimicrobial resistance reservoir is present in primary care patients.

DOI: <https://doi.org/10.1089/mdr.2013.0013>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-87553>

Journal Article

Published Version

Originally published at:

Nüesch-Inderbinen, Magdalena; Abgottspon, Helga; Zurfluh, Katrin; Nüesch, Hans-Jakob; Stephan, Roger; Hächler, Herbert (2013). Cross-sectional study on fecal carriage of Enterobacteriaceae with resistance to extended-spectrum cephalosporins in primary care patients. *Microbial Drug Resistance*, 19(5):362-369.

DOI: <https://doi.org/10.1089/mdr.2013.0013>

Cross-Sectional Study on Fecal Carriage of *Enterobacteriaceae* with Resistance to Extended-Spectrum Cephalosporins in Primary Care Patients

Magdalena T. Nüesch-Inderbilen, Helga Abgottspon, Katrin Zurfluh, Hans J. Nüesch, Roger Stephan, and Herbert Hächler

The aim of this study was to gain knowledge of the local epidemiology of extended-spectrum cephalosporin-resistant bacteria in primary care patients in a Swiss community. Fecal swabs were obtained from 291 primary care patients. Phenotyping and genotyping methods were used for further characterization of the isolates. Risk factors associated with carriage of β -lactam-resistant strains were determined. Extended-spectrum cephalosporin-resistant *Enterobacteriaceae* were detected in 15 (5.2%) of the primary care patients. Thirteen isolates were CTX-M producers, one produced SHV-12, and three carried CMY-2. The pathogenic pandemic clone *Escherichia coli* ST131 was detected in 26.6% of the patients. Two patients (13.3%) carried two distinct strains simultaneously. There was a statistically significant risk of carriage of resistant strains for persons with a history of antibiotic therapy 4 months before sampling ($p=0.05$), markedly for therapy with β -lactam ($p=0.01$). Age, gender, or history of hospitalization 4 months before sampling was not a risk factor for the acquisition of resistant bacteria in the analyzed patients. The relatively low prevalence of extended-spectrum cephalosporin-resistant strains in the community reflects the nationwide restrictive policy of antibiotic prescription as well as local implementation thereof. Nevertheless, our study shows that a potent antimicrobial resistance reservoir is present in primary care patients.

Introduction

THE MOST IMPORTANT mechanism of antibiotic resistance in *Enterobacteriaceae* is based on the production of enzymes that inactivate β -lactam antibiotics, including cephalosporins and monobactams by hydrolyzing their β -lactam ring. These so-called extended-spectrum β -lactamases (ESBLs) are usually acquired by horizontal gene transfer and have been detected in human clinical isolates of *Enterobacteriaceae* since the early 1990s,²⁴ originally as plasmid-mediated point mutational derivatives of the TEM- and SHV- β -lactamase families, and then increasingly, as environmental originating CTX-M enzymes,¹⁵ or other less frequent ESBLs, such as OXA- or PER-ESBLs.^{6,10} In addition, plasmid-mediated AmpC-type β -lactamases (pAmpCs) are increasingly reported worldwide, representing a new threat to successful antibiotic therapy because they are not, like ESBLs, susceptible to β -lactamase inhibitors such as clavulanic acid or sulbactam, and they possess a wider spectrum of the enzymatic activity.¹⁶

As a further matter of concern, resistance caused by ESBLs or pAmpCs is often associated with resistance to other clas-

ses of antibiotics such as fluoroquinolones, aminoglycosides, and sulfamethoxazole/trimethoprim, resulting in multidrug-resistant strains.¹⁰

Infections due to multidrug-resistant, ESBL-producing, Gram-negative bacteria are treated with carbapenems (e.g., imipenem, ertapenem, or meropenem), which have become invaluable antimicrobial drugs of last resource for preventing and treating many life-threatening nosocomial infections.²² Therefore, it is most worrisome to note the emergence and global dissemination of carbapenem-hydrolyzing β -lactamases in Gram-negative bacteria, including *Enterobacteriaceae* and nonfermenters.² Unlike the ESBL- and AmpC- β -lactamases, which can be readily classified according to their molecular³ or functional properties,⁶ carbapenamases represent a diverse group of enzymes and remain notoriously difficult to detect. Hence, the actual prevalence of carbapenemase-producing strains remains uncertain.²²

Enterobacteriaceae, especially *Escherichia coli*, are key indicator organisms for detecting the occurrence of ESBLs in humans, animals, and the environment. The rapid dissemination of different β -lactamases in clinically important

National Centre for Enteropathogenic Bacteria and Listeria, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.

bacteria is a serious threat to public health. Local knowledge of the epidemiology and characterization of resistant strains is of growing importance. The aim of the present study was therefore (1) to assess the occurrence of resistance to extended-spectrum cephalosporins in *Enterobacteriaceae* harbored by a collective of primary care patients in a suburban community in Switzerland, (2) to characterize such isolates by antibiotic susceptibility testing, identification of the *bla* genes, multilocus sequence typing (MLST), and determination of phylogenetic groups of *E. coli*, and (3) determination of risk factors for fecal carriage of resistant strains.

Materials and Methods

Sample collection

This cross-sectional study constituted a convenience sample of 291 primary care patients reporting to their general practitioner in a suburban community in the greater area of Zürich, Switzerland, during a period of 7 weeks in May and June 2012, for medical consultation. Fecal swabs were obtained from adult patients between 23 and 96 years of age.

Each patient was fully informed of the nature of the sampling and consent was obtained orally from each individual during their consultation with the doctor and before sampling. Minors and nonregistered patients were excluded from participation and each patient was sampled once only, by the practitioner, using a commercial rectal swab tube containing the Amies transport medium (Copan).

Swabs were numbered consecutively. Each anonymised number was supplemented with the patient's age, gender, histories of hospitalization, and antibiotic therapies during the 4 months before sampling, and notable comorbidities.

The study was approved by the local ethics committee of Zürich and is registered as number KEK-StV-Nr. 54/12.

Phenotypical detection of strains resistant to extended-spectrum cephalosporins and antimicrobial susceptibility testing

Each swab was incubated for 24 hr at 37°C in 10 ml of the EE Broth (BD) for enrichment. One loopful each of the enrichment cultures was inoculated onto chromogenic Brilliance ESBL agar and Brilliance CRE agar (Oxoid) and incubated at 37°C for 24 hr under aerobic conditions. All colonies with different chromatism and morphology were picked from the selective plates and subcultured on sheep blood agar (Difco laboratories; 5% sheep blood, SB055, Oxoid) at 37°C for 24 hr. Isolates from the same patient displaying different colony colorations and morphologies were included. Oxidase-negative isolates were subjected to identification by API ID 32 E (bioMérieux).

Disk susceptibility testing and interpretation were performed using antibiotic disks (Becton Dickinson and Company), according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI)⁹ as well as Etest ESBL strips (bioMérieux).

Characterization of *bla* genes

The isolates were analyzed by screening for *bla* genes. DNA was extracted by a standard heat lysis protocol. Thereafter, specific primer sets (custom-synthesized by Mi-

croSynth) were used to amplify enterobacterial β -lactamase-encoding genes belonging to *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX-M}¹⁴ and *bla*_{CMY-2}^{5,12} the last set supplemented with the following newly designed primer *cmv-dn-3* from the *bla*_{CMY-2} downstream flanking region: 5'ATGCGCATGGGATTTTCCTTGC3'. Resulting amplicons were purified using the PCR Purification Kit (QIAGEN) according to the manufacturer's recommendations. Custom sequencing was performed by Microsynth and the nucleotide and protein sequences were analyzed with Codon Code Aligner V. 3.7.1.1. For database searches, the BLASTN program of NCBI (www.ncbi.nlm.nih.gov/blast) was used.

Multilocus sequence typing of β -lactamase-producing *E. coli*

Internal fragments of seven housekeeping genes (*adh*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) were sequenced³⁰ and alleles and sequence types (ST) were assigned in accordance with the *E. coli* MLST website (<http://mlst.ucc.ie/>).

Determination of phylogenetic groups in β -lactamase-producing *E. coli*

After DNA extraction using a standard heat lysis protocol, β -lactamase-producing *E. coli* isolates were classified by phylogenetic PCR into four main groups (A, B1, B2, and D), as described previously.⁸

Statistical analysis of anonymised patients' data

Risk factors for fecal carriage were calculated based on the provided data regarding the patients' history (age, gender, history of hospitalization 4 months before sampling, application of antibiotics, and history of diverticulitis).

Patients were stratified into three age groups: group 1, 20–40 years; group 2, 40–60 years; and group 3, >60 years. Data on antibiotic therapies were categorized according to antibiotic classes and, in the case of β -lactams, additionally subdivided into their subgroups.

Statistical values were calculated using the Fischer's one-tailed exact test. Statistical significance was set at $p \leq 0.05$.

Results

Study population

The total number of primary care patients included in this analysis was 291, with a male/female ratio of (165/126), a mean age of 64.5 years, and a median age of 66 years. Age ranged from 23 to 96 and comprised the following groups: group 1, 20–40 years ($n=20$); group 2, 40–60 years ($n=85$); and group 3 ($n=186$). Thirty-four patients had been hospitalized at some point during 4 months before participation in the study. Thirty patients had received antibiotic therapy, predominantly amoxicillin/clavulanic acid (20 patients), prescribed by their general practitioner during the same time scale. Fourteen (4.8%) of the patients had a comorbidity of confirmed diverticulitis.

Extended-spectrum cephalosporin-resistant *Enterobacteriaceae* were detected in 15 (5.2%; 9 male/6 female) of the primary care patients. Multiple strains were isolated from fecal swabs of 2 patients, one simultaneously carrying two distinct *E. coli* strains, and one an *E. coli* and a *Citrobacter youngae* strain.

TABLE 1. RISK FACTORS INFLUENCING FECAL CARRIAGE OF EXTENDED-SPECTRUM CEPHALOSPORIN-RESISTANT ISOLATES IN PRIMARY CARE PATIENTS

Characteristic	No. of patients	No. of patients identified as carriers	OR (95% CI)	p-Value ^a
Age groups of screened patients				
Age group 20–40	20	3	3.8 (1.0–14.8)	0.07
Age group 40–60	85	2	0.4 (0.1–1.6)	0.1
Age group >60	186	10	1.1 (0.4–3.4)	0.5
Gender				
Male	165	9	1.2 (0.4–3.3)	0.5
Female	126	6	0.9 (0.3–2.5)	0.5
History of hospitalization 4 months before sampling	34	3	2.0 (0.5–7.4)	0.2
History of antibiotic therapy 4 months before sampling	30	4	3.5 (1.1–11.8)	0.05
Prescribed antibiotic classes 4 months before sampling				
β-lactams	20	4	5.9 (1.7–20.6)	0.01
Macrolides	7	1	3.2 (0.4–28.6)	0.3
Quinolones	4	1	6.5 (0.6–66.5)	0.2
Sulfonamides	1	0	0	0.9
Tetracyclines	1	0	0	0.9
Fosfomycins	1	1	Infinity	
Nitroimidazoles	1	0	0	0.9
Prescribed β-lactam antibiotics 4 months before sampling				
Amoxicillin	2	1	19.6 (1.2–330.7)	0.1
Amoxicillin & clavulanic acid	14	3	6.0 (1.5–24.5)	0.02
1st generation cephalosporin	1	0	0	0.9
2nd generation cephalosporin	2	0	0	0.9
3rd generation cephalosporin	1	1	Infinity	
History of diverticulitis	14	2	3.4 (0.7–16.7)	0.2

^aFisher's exact probability test (one-tailed); entries for which the *p*-values were ≤0.05 are in bold type. OR, odds ratio; CI, confidence interval.

Risk factors for fecal carriage were calculated based on the provided data regarding the patients' history and are summarized in Table 1. Thereby, previous therapy with the combination compound amoxicillin/clavulanic acid emerged as the only significant risk factor for subsequent colonization with cephalosporin-resistant isolates (*p*=0.02). Age, gender, or history of hospitalization 4 months before sampling was not a risk factor. Furthermore, diverticulitis as a risk factor could not be established.

Antimicrobial susceptibility patterns of resistant isolates

From 291 fecal swabs, 17 isolates were analyzed. Their identities and antimicrobial susceptibility profiles are shown in Table 2. When applying CLSI criteria, 9 of 17 isolates (53%) were resistant to the 3rd generation cephalosporin cefotaxime in the disk diffusion test. Elevated minimal inhibitory concentrations (MIC) to cefotaxime and the 4th generation cephalosporin cefepime were observed in 70% and 17.6% of the *Enterobacteriaceae*, respectively, when the E test was utilized. All isolates displaying low MIC values to the carbapenem antibiotic imipenem.

Coresistance to antibiotics other than β-lactams was frequent, with 47% resistant to quinolones, 29% to gentamicin, 35% to kanamycin and, ranking highest in the class of the aminoglycosides, 53% to streptomycin. Tetracycline resistance was detected in 70.6%, and chloramphenicol resistance in 12% of the strains. The highest rate of resistance was to sulfamethoxazole, with 82% affected, and followed by 65% of trimethoprim-resistant isolates.

Distribution of β-lactamase genes

The identification of the isolates' *bla* genes are summarized in Table 3. Of the detected CTX-M enzymes, seven belonged to the CTX-M group 1 (to which CTX-M-1 and CXT-M-15 belong) and six belonged to the CTX-M group 9 (to which CTX-M-14 and CTX-M-27 belong), according to the classification of Bonnet.⁴ In total, 13 (76%) of the analyzed isolates harbored a CTX-M β-lactamase (Table 3). The most frequently detected CTX-M enzyme was CTX-M-15, which was found in four *E. coli* and one *C. youngae* isolate, giving rise to a prevalence of CTX-M-15 in the analyzed *Enterobacteriaceae* isolates of 31.3%. CTM-X-15 producers displayed the widest range of coresistances, compared to other isolates. CTX-M-1 was detected in two *E. coli* strains (12.5% of the *E. coli* isolates). Three *E. coli* (18.8%) harbored CTX-M-14. Three further *E. coli* isolates (18.8%) produced CTX-M-27.

The pAmpC-β-lactamase CMY-2 was detected in three *E. coli* strains (18.8%). These strains displayed few to no coresistances. SHV-12 was detected in one *E. coli* strain (6.3%).

Two strains in our study were detected together as multiple isolates from single patients. One patient was shown to be a carrier of an *E. coli* producing CTX-M-14 together with a CTX-M-15 producing *C. youngae*. Simultaneous carriage of two distinct *E. coli* strains producing CMY-2 was noted from a further patient.

ST distribution of *E. coli* isolates

MLST of the *E. coli* isolates revealed nine different STs (Table 3). Most isolates belonged to ST131 (four isolates,

TABLE 2. ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF EXTENDED-SPECTRUM CEPHALOSPORIN-RESISTANT ISOLATES FROM FECAL SAMPLES OF PRIMARY CARE PATIENTS

Strain	Species	Disk diffusion test [mm]													Etest [µg/ml]						
		AM	AMC	CF	CTX	CIP	NA	GM	K	S	TE	C	SMZ	TMP	IP	CT	CTL	TZ	TZL	PM	PML
HC11	<i>Escherichia coli</i>	10	19	6	23	34	24	20	20	9	6	27	6	6	0.125	<0.25	0.032	12	0.094	<0.025	<0.064
HC14B	<i>E. coli</i>	6	16	6	12	30	24	22	6	6	6	26	6	6	0.19	>16	0.016	1	<0.064	12	<0.064
HC14G	<i>Citrobacter youngae</i>	6	17	6	10	6	6	8	12	13	6	25	6	6	0.125	>16	0.023	4	<0.064	>16	<0.064
HC22E	<i>E. coli</i>	6	10	6	12	30	25	21	22	17	21	26	12	27	0.25	>16	>1	>32	>4	0.38	0.125
HC22C	<i>E. coli</i>	6	12	6	17	6	6	21	21	16	22	28	16	6	0.19	>16	>1	>32	>4	<0.25	0.094
HC56	<i>E. coli</i>	6	21	6	15	6	6	23	22	18	20	30	17	28	0.25	>16	0.016	1.5	<0.064	0.75	<0.064
HC111	<i>E. coli</i>	6	23	6	15	6	6	19	19	10	6	27	6	6	0.19	>16	0.016	1.5	<0.064	1	<0.064
HC121	<i>E. coli</i>	6	18	6	12	6	6	8	9	8	6	29	6	6	0.19	>16	0.016	6	<0.064	3	<0.064
HC122	<i>E. coli</i>	6	16	6	14	6	6	7	13	14	6	28	6	6	0.19	>16	0.016	6	<0.064	3	<0.064
HC171	<i>E. coli</i>	6	17	6	18	6	6	20	11	13	6	28	6	6	0.19	1.5	0.023	<0.5	<0.064	<0.25	<0.064
HC192	<i>E. coli</i>	6	22	6	15	33	22	19	20	17	6	27	6	27	0.25	8	<0.016	<0.5	<0.064	1	<0.064
HC200	<i>E. coli</i>	6	19	6	7	6	6	19	19	6	6	6	6	27	0.125	>16	0.032	16	0.094	>16	<0.064
HC247	<i>E. coli</i>	6	19	6	14	26	24	9	6	6	6	6	6	25	0.125	>16	0.016	<0.5	<0.064	3	<0.064
HC264	<i>E. coli</i>	6	20	6	9	30	26	18	18	6	6	25	6	6	0.125	>16	0.023	3	0.064	3	<0.064
HC267	<i>E. coli</i>	6	20	6	17	25	8	8	18	10	6	26	6	6	0.125	8	<0.016	<0.5	<0.064	1	<0.064
HC270	<i>E. coli</i>	6	22	6	14	28	25	18	19	10	22	25	6	6	0.19	>16	0.016	0.5	<0.064	1.5	<0.064
HC281E	<i>E. coli</i>	6	14	6	19	34	29	18	19	15	22	25	20	26	0.125	6	>1	12	>4	<0.064	<0.064

Resistance to specific antibiotics are in bold type.

AM, ampicillin; AMC, amoxicillin-clavulanic acid; CF, cephalothin; CTX, cefotaxime; CIP, ciprofloxacin; NA, nalidixic acid; GM, gentamicin; K, kanamycin; S, streptomycin; TE, tetracycline; C, chloramphenicol; SMZ, sulfamethoxazole; TMP, trimethoprim; IP, imipenem; CT, ceftazidime; CTL, ceftazidime/clavulanic acid; TZ, ceftazidime/clavulanic acid; PM, ceftipime; PML, ceftipime/clavulanic acid.

TABLE 3. FURTHER CHARACTERISTICS OF EXTENDED-SPECTRUM CEPHALOSPORIN-RESISTANT ISOLATES FROM FECAL SWABS OF PRIMARY CARE PATIENTS

Strain	Species	β-lactamase	MLS T ^b	Phylogenetic group	Patient data ^c			Coreistance
					Age	Gender	Hospitalization	
HC11	<i>E. coli</i>	SHV-12	ST2142	A	70	M		S, TE, SMZ, TMP
HC14B ^a	<i>E. coli</i>	CTX-M-14	ST131	B2				K, S, TE, SMZ, TMP
HC14G ^a	<i>C. youngae</i>	CTX-M-15	NA	nd	37	M	Yes	CIP, NA, GM, K, TE, SMZ, TMP
HC22E ^a	<i>E. coli</i>	CMY-2	ST2142	D				SMZ
HC22C ^a	<i>E. coli</i>	CMY-2	ST448	B1	46	F	Yes	CIP, TMP
HC56	<i>E. coli</i>	CTX-M-27	ST131	B2	57	M		CIP, NA,
HC111	<i>E. coli</i>	CTX-M-27	ST131	B2	71	M		CIP, NA, S, TE, SMZ, TMP
HC121	<i>E. coli</i>	CTX-M-15	ST90	A	23	M		CIP, NA, GM, K, S, TE, SMZ, TMP
HC122	<i>E. coli</i>	CTX-M-15	ST90	A	89	M	yes	CIP, NA, GM, K, TE, SMZ, TMP
HC171	<i>E. coli</i>	CTX-M-15	ST131	B2	37	F	Yes	CIP, NA, K, TE, SMZ, TMP
HC192	<i>E. coli</i>	CTX-M-1	ST10	A	62	F		TE, SMZ
HC200	<i>E. coli</i>	CTX-M-15	ST405	D	68	M		CIP, NA, S, TE, C, SMZ,
HC247	<i>E. coli</i>	CTX-M-14	ST362	D	80	F	Yes	K, S, TE, C, SMZ
HC264	<i>E. coli</i>	CTX-M-27	ST2142	D	65	M		S, TE, SMZ, TMP
HC267	<i>E. coli</i>	CTX-M-14	ST10	A	87	M		S, TE, SMZ, TMP
HC270	<i>E. coli</i>	CTX-M-1	ST962	B1	63	F		S, SMZ, TMP
HC281E	<i>E. coli</i>	CMY-2	ST814	nd ^d	80	F	yes	None

^aStrains isolated from one patient each, respectively.

^bMultilocus sequence type.

^cHistories of hospitalization and antibiotic therapies during the 4 months before sampling.

^d*E. coli* HC281E could not be assigned to any of the major phylogenetic groups tested.

NA, not applicable; nd, not determined; ST, sequence type.

25%) followed by ST2142 (three isolates, 18.8%). Two isolates belonged to ST90 (12.5%). Other STs, including the pandemic clone ST405¹¹ were detected as singletons.

Phylogenetic groups of *E. coli* isolates

Phylogenetic grouping categorized 50% of the *E. coli* strains as belonging to the extraintestinal pathogenic group B2 (four strains) or D (four strains), as shown in Table 3. The other 50% of the isolates belonged to commensal groups A (five strains) and B1 (two strains). One strain, HC281E, could not be assigned to any of the tested phylogenetic groups.

All four ST131 isolates belonged to the virulent extraintestinal phylogenetic group B2. The pandemic clone ST405, as well as two ST2142 isolates and the isolate ST362 belonged to the phylogenetic group D.

Both ST90 and ST10 isolates belonged to the commensal phylogenetic group A, as did one ST2142 isolate. Two further isolates, ST448 and ST962, were assigned to the commensal phylogenetic group B1.

No clonal relationship among β -lactamase producers

CTX-M enzymes were found among a large number of clonal types (Table 3). CTX-M group 1 enzymes (detected in seven strains) were in one case (isolate HC171) harbored by pandemic clone ST131-B2 (CTX-M-15) and in one further instance (isolate HC200) by pandemic clone ST405-D (CTX-M-15). All other enzymes of this group were detected in commensal *E. coli* strains, or, as in one case, in a *C. youngae* (isolate HC14G).

CTX-M group 9 enzymes (from six strains) were detected in three pandemic ST131-B2 clones harboring CTX-M-14 in one case, and CTX-M-27 in two cases (isolate HC14B, HC56, and HC111, respectively). One CTX-M-14 and one CTX-M-27 enzyme were detected in extraintestinal pathogenic phylogenetic group D strains (isolates HC247 and HC264, respectively). One CTX-M-14 was harbored by a commensal *E. coli* strain (HC267).

Two CMY-2-producing *E. coli* strains isolated simultaneously from the same patient belonged to the extraintestinal pathogenic phylogenetic group D (isolate HC22E) and to commensal group B1 (isolate HC22C), respectively. One *bla*_{CMY-2} gene was found in an *E. coli* isolate, typed as ST814, which did not belong to any of the tested phylogenetic groups (isolate HC281E). SHV-12 was detected in a commensal *E. coli* strain (isolate HC11).

Discussion

One of the major challenges public healthcare systems face today is the global spread of extended-spectrum cephalosporin-resistant Gram-negative bacteria from the hospital setting into the community, necessitating collaborative approaches to antimicrobial surveillance.

An important strategy for monitoring and controlling the spread of multidrug-resistant pathogens is the determination of their occurrence in community-based patients. Therefore, knowledge of the local epidemiology and risk factors is crucial to estimate the magnitude of the problem.

The fecal carriage rate of extended-spectrum cephalosporin-resistant *Enterobacteriaceae* in Swiss primary care patients is 5.2%. This is slightly lower than the rate of 5.8% ESBL-

producing *Enterobacteriaceae* found in a collective of healthy factory staff in Switzerland,¹⁴ possibly reflecting local or behavioral differences, such as traveling, which is a known risk factor for acquiring ESBL-producing *E. coli*.²⁷ Carriage rates are lower than those found in primary care patients in Belgium,²⁰ the Netherlands,²⁵ and Great Britain,²⁹ where ESBL-producing *Enterobacteriaceae* were detected in 7%, 10%, and 11% of community-based patients, respectively. For the Netherlands, a country comparably restrictive in its antibiotic prescription policy²⁵ such as Switzerland,¹³ the unexpectedly high rate of carriage seems to be linked to the country's high antibiotic use in the poultry industry and transmission through the food chain.¹⁸

Although Switzerland compares relatively favorable to other European countries, our results show that ESBL-producing bacteria are indeed present in the community, a fact that should be taken into account when considering treatment options.

In our study, the CTX-M enzymes were the most prevalent ESBLs. This is consistent with the dissemination noted for other European countries, with CTX-M-15 the most common.^{7,19}

The global distribution of CTX-M-15-producing *E. coli* is mostly due to the worldwide pandemic uropathogenic clone ST131-B2,²⁶ and, to a lesser extent, clone ST405-D.¹¹ However, the CTX-M-15-producing strains identified in this study belonged to multiple STs and to diverse, commensal as well as pathogenic phylogenetic groups, or, as in one case, even to another species *C. youngae*. Together with the inhomogenous antibiotic susceptibility patterns of the individual isolates, one must assume that the dissemination of CTX-M β -lactamases in the community is driven primarily by the spread of mobile genetic elements, rather than by clonal dissemination.

Although the finding of 26.6% of clone ST131 in our patient collective comes close to the rate of previous reports showing 24%,²⁹ we found that *E. coli* ST131 strains isolated from our collective were, as opposed to previous reports, not predominantly CTX-M-15 producers, but harbored by the majority the group 9 enzymes CTX-M-14 and CTX-M-27. Interestingly, *E. coli* ST131 harboring CTX-M-27 in humans are rare in Europe,²¹ but have been detected in tertiary care patients in Israel,¹ and a very recent study from Japan reported for the first time a cluster of CTX-M-27-producing *E. coli* among clinical isolates,¹⁷ suggesting that this clone is currently emerging.

At present, pAmpC β -lactamases are being detected globally with the CMY-2 β -lactamase being the most widely distributed in *Enterobacteriaceae*.¹⁶ Three of the strains analyzed in our study harbored CMY-2. All demonstrated clonal diversity, again suggesting a dissemination of mobile genetic elements within the community. This is particularly suggestive in the case of a patient with a history of hospitalization exhibiting coexistence of clonally distinct CMY-2-producing strains, where horizontal transfer of the *bla*_{CMY-2} gene appears to have occurred between the hospital-associated pathogenic phylogenetic group D *E. coli* and the commensal group B1 strain.

SHV-12, originally detected in an *E. coli* hospital strain in Switzerland²³ has disseminated to commensal *E. coli* strains, and although rare, appears to persist within the community. This finding provides further evidence for the propagation of a resistance gene pool in nonpathogenic bacteria in the population.

None of the analyzed strains contained any of the currently globally disseminating and clinically important carbapenem hydrolyzing metallo- β -lactamases (MBLs) such as the active-on-imipenem (IMP) type, the verona-integron-encoded MBL (VIM) type,²⁸ or the New-Delhi MBL (NDM) type of MBLs.²²

This study offers insight into the antibacterial resistance gene pool not only in bacterial pathogens, but in opportunistic pathogens and commensal bacteria of the Swiss population. The impact of these potent reservoirs of resistance genes on public health is not yet sufficiently investigated and calls for collaborative approaches by the scientific and medical community.

Acknowledgments

This project was partially funded by the Swiss Federal Office of Public Health.

Disclosure Statement

All authors disclose no commercial associations that might create a conflict of interest in connection with this study.

References

- Adler, A., M. Gniadkowski, A. Baraniak, R. Izdebski, J. Fiett, W. Hryniewicz, S. Malhotra-Kumar, H. Goossens, C. Lammens, and Y. Lerman. 2012. Transmission dynamics of ESBL-producing *Escherichia coli* clones in rehabilitation wards at a tertiary care centre. *Clin. Microbiol. Inf.* 8:497–505.
- Akova, M., G.L. Daikos, L. Tzouveleakis, and Y. Carmeli. 2012. Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. *Clin. Microbiol. Infect.* 18:439–448.
- Ambler, R.P., A.F. Coulson, J.M. Frère, J.M. Ghuysen, B. Joris, M. Forsman, R.C. Levesque, G. Tiraby, and S.G. Waley. 1991. A standard numbering scheme for the class A beta-lactamases. *Biochem. J.* 276:269–272.
- Bonnet, R. 2004. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. *Antimicrob. Agents Chemother.* 48:1–14.
- Briñas, L., M.A. Moreno, M. Zarazaga, C. Porrero, Y. Sáenz, M. García, L. Dominguez, and C. Torres. 2003. Detection of CMY-2, CTX-M-14, and SHV-12 β -lactamases in *Escherichia coli* fecal-sample isolates from healthy chickens. *Antimicrob. Agents Chemother.* 47:2056–2058.
- Bush, K., and G.A. Jacoby. 2010. Updated functional classification of beta-lactamases. *Antimicrob. Agents Chemother.* 54:969–976.
- Cantón, R., and T.M. Coque. 2006. The CTX-M β -lactamase pandemic. *Curr. Opin. Microbiol.* 9:466–475.
- Clermont, O., S. Bonacorsi, and E. Bingen. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl. Environ. Microbiol.* 66:4555–4558.
- Clinical and Laboratory Standards Institute. 2008. Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement. Document M100-S18. Wayne, PA: Clinical and Laboratory Standards Institute.
- Coque, T.M., F. Baquero, and R. Canton. 2008. Increasing prevalence of ESBL-producing *Enterobacteriaceae* in Europe. *Euro. Surveill.* 13:1–11.
- Coque, T.M., A. Novais, A. Carattoli, L. Poirel, J. Pitout, L. Peixe, F. Baquero, R. Cantón, and P. Nordmann. 2008. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. *Emerg. Infect. Dis.* 14:195–200.
- Endimiani, A., A. Rossano, D. Kunz, G. Overesch, and V. Perreten. 2012. First countrywide survey of third-generation cephalosporin-resistant *Escherichia coli* from broilers, swine, and cattle in Switzerland. *Diagn. Microbiol. Infect. Dis.* 73:31–38.
- Filippini, M., G. Masiero, and K. Moschetti. 2006. Socio-economic determinants of regional differences in outpatient antibiotic consumption: evidence from Switzerland. *Health Policy* 78:77–92.
- Geser, N., R. Stephan, B.M. Korczak, L. Beutin, and H. Hächler. 2012. Molecular identification of extended-spectrum- β -lactamase genes from *Enterobacteriaceae* isolated from healthy human carriers in Switzerland. *Antimicrob. Agents Chemother.* 56:1609–1612.
- Hawkey, P.M., and A.M. Jones. 2009. The changing epidemiology of resistance. *J. Antimicrob. Chemother.* 64:3–10.
- Jacoby, G.A. 2009. AmpC beta-lactamases. *Clin. Microbiol. Rev.* 22:161–182.
- Kuroda, H., H. Yano, Y. Hirakata, K. Arai, S. Endo, H. Kanamori, H. Yamamoto, S. Ichimura, M. Ogawa, M. Shimojima, M. Komatsu, T. Jonai, S. Itagaki, Y. Nonomiya, A. Suwabe, and M. Kaku. 2012. Molecular characteristics of extended-spectrum β -lactamase-producing *Escherichia coli* in Japan: emergence of CTX-M-15-producing *E. coli* ST131. *Diagn. Microbiol. Infect. Dis.* 74:201–203.
- Leverstein-van Hall, M.A., C.M. Dierikx, J. Cohen Stuart, G.M. Voets, M.P. van den Munkhof, A. van Essen-Zandbergen, T. Platteel, A.C. Fluit, N. van de Sande-Bruinsma, J. Scharinga, M.J. Bonten, D.J. Mevius; National ESBL surveillance group. 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin. Microbiol. Infect.* 17:873–880.
- Livermore, D.M., R. Cantón, M. Gniadkowski, P. Nordmann, G.M. Rossolini, G. Arlet, J. Ayala, T.M. Coque, I. Kern-Zdanowicz, F. Luzzaro, L. Poirel, and N. Woodford. 2007. CTX-M: changing the face of ESBLs in Europe. *J. Antimicrob. Chemother.* 59:165–174.
- Meex, C., P. Melin, J.D. Docquier, T. Kabasele, P. Huynen, P.M. Tulkens, D. Giet, and P. De Mol. 2008. Presence of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in the faecal flora of patients from general practice. Abstract presented at the 18th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Barcelona, April 19–22. Abstract no. P631.
- Nicolas-Chanoine, M.H., V. Jarlier, J. Robert, G. Arlet, L. Drieux, V. Leflon-Guibout, C. Laouénan, B. Larroque, V. Caro, F. Mentré; Group Coli β . 2012. Patient's origin and lifestyle associated with CTX-M-producing *Escherichia coli*: a case-control-control study. *PLoS One* 7:e30498.
- Nordmann, P., T. Naas, and L. Poirel. 2011. Global spread of Carbapenemase-producing *Enterobacteriaceae*. *Emerg. Infect. Dis.* 17:1791–1798.
- Nüesch-Inderbinnen, M.T., F.H. Kayser, and H. Hächler. 1997. Survey and molecular genetics of SHV beta-lactamases in *Enterobacteriaceae* in Switzerland: two novel enzymes, SHV-11 and SHV-12. *Antimicrob. Agents Chemother.* 41:943–949.
- Paterson, D.L., and R.A. Bonomo. 2005. Extended-spectrum β -lactamases: a clinical update. *Clin. Microbiol. Rev.* 18:657–686.
- Reuland, E.A., I.T. Overdevest, N. Al Naiemi, J.S. Kalpoe, M.C. Rijnsburger, S.A. Raadsen, I. Ligtenberg-Burgman, K.W. van der Zwaluw, M. Heck, P.H. Savelkoul, J.A.

- Kluytmans, and C.M. Vandenbroucke-Grauls. 2012. High prevalence of ESBL-producing *Enterobacteriaceae* carriage in Dutch community patients with gastrointestinal complaints. *Clin. Microbiol. Infect.* [Epub ahead of print]; DOI: 10.1111/j.1469-0691.2012.03947.
26. Rogers, B.A., H.E. Sidjabat, and D.L. Paterson. 2011. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. *J. Antimicrob. Chemother.* **66**:1–14.
27. Tängdén, T., O. Cars, A. Melhus, and E. Löwdin. 2010. Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. *Antimicrob. Agents Chemother.* **54**:3564–3568.
28. Walsh, T.R., M.A. Toleman, L. Poirel, and P. Nordmann. 2005. Metallo-beta-lactamases: the quiet before the storm? *Clin. Microbiol. Rev.* **18**:306–325.
29. Wickramasinghe, N.H., L. Xu, A. Eustace, S. Shabir, T. Saluja, and P.M. Hawkey. 2012. High community faecal carriage rates of CTX-M ESBL-producing *Escherichia coli* in a specific population group in Birmingham, UK. *J. Antimicrob. Chemother.* **67**:1108–1113.
30. Wirth, T., D. Falush, R. Lan, F. Colles, P. Mensa, L.H. Wieler, H. Karch, P.R. Reeves, M.C. Maiden, H. Ochman, and M. Achtman. 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol. Microbiol.* **60**:1136–1151.

Address correspondence to:

Roger Stephan, DVM

National Centre for Enteropathogenic Bacteria and Listeria

Institute for Food Safety and Hygiene

Vetsuisse Faculty

University of Zurich

Winterthurerstrasse 272

8057 Zurich

Switzerland

E-mail: stephanr@fsafety.uzh.ch